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OBSERVATIONS ON KARYOKINESIS

I N

S P I R O G Y R A.

BY

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OBSERVATIONS ON KARYOKINESIS IN SPIROGYRA.

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INTRODUCTION. Some time ago I was seeking for a method, by which it would be possible to make sections of very small Algae and similar objects with the same precision, obtainable in the case of larger plants. From a methodical point of view my success till now was but an indifferent one. But as my experiments were chiefly made with a large species of Spirogyra, many nuclei and cells of which were in a state of division, I gradually obtained a certain amount of observations on the structure of the nuclei and their process of division in this plant.

These observations I enlarged from time to time and as they seem to me not wholly without interest, I will now give the results to the public.

Several species of Spirogyra have attracted the attention of many eminent observers. The phenomena of their nuclear and cell-division have been studied by STRASBURGER ¹⁾, MACFARLANE ²⁾, FLEMING ³⁾, TANGL ⁴⁾, MEUNIER ⁵⁾, and BEHRENS ⁶⁾; the structure of the nucleoli in the resting nuclei moreover by ZACHARIAS ⁷⁾, and indeed

¹⁾ STRASBURGER. Zellbildung u. Zelltheilung. 3. Ed. 1880; Ueb. den Theilungsvorgang der Zellkerne. Archiv. f. Mikrosk. Anat. Vol. 21, 1882, p. 476; die Controversen der indirecten Kerntheilung. Archiv. f. Mikrosk. Anat. Vol. 23, 1884; Ueb. Kern- u. Zelltheilung. 1888.

²⁾ MACFARLANE. The structure and division of the vegetable cell. Trans. Bot. Soc. of Edinburgh. Vol. XIV, 1881, p. 192.

³⁾ FLEMING. Zellsubstanz, Kern- u. Zelltheilung, 1882.

⁴⁾ TANGL. Ueb. die Theilung der Kerne in Spirogyra-Zellen. Sitzb. d. K. Akad. d. Wiss. in Wien, I. Abth. Vol. 85, 1882, p. 268.

⁵⁾ MEUNIER. Le nucléole des Spirogyra. La cellule, Vol. III. p. 333.

⁶⁾ BEHRENS. Zur Kenntniss einiger Wachstums- und Gestaltungsvorgänge in der vegetabilischen Zelle. Bot. Ztg. Vol. 48, 1890, p. 81.

⁷⁾ ZACHARIAS. Ueb. den Nucleolus. Bot. Ztg. Vol. 43, 1885, p. 257.

our knowledge may be regarded in many respects as very accurate and even complete.

I will first call to the reader's mind some of the principal facts, recorded by the botanists mentioned above, and especially direct his attention to those points, over which my researches seem to shed some additional light.

Structure of the resting nucleus. A nuclear membrane has been observed by all investigators and delineated by FLEMMING, STRASBURGER and others.

The nuclear plasm within this membrane has been represented by FLEMMING as a network, consisting of achromatic substance¹⁾, MEUNIER has in many of his figures done the same, though he draws the meshes somewhat narrower, and STRASBURGER's²⁾ figures agree in most particulars with those of FLEMMING, though he describes the structure of the nuclear plasm as consisting of one or more greatly coiled threads. The number of nucleoli, observed in the resting nucleus, is one or two, and all investigators have remarked, that these little bodies are much more tenacious of nuclear dyes than the surrounding nuclear plasm. In this last is found at best but a very small number of little chromatic granules³⁾ and MEUNIER goes so far as to deny even the existence of these⁴⁾. At all events a nuclear stain has the same effect upon the nucleoli, which it exercises in the division-stages upon the nuclear segments, whilst this testimony can in no wise be given of the nuclear plasm.

Not many observers have studied the finer structure of the nucleolus. TANGL has remarked, that it possesses a well-defined membrane⁵⁾ and MEUNIER has done the same⁶⁾.

The contents of this membrane are by most authors represented as a homogeneous mass, but one of FLEMMING's figures⁷⁾ shows, that this is not always the case and that it can contain some more transparent spots.

Several drawings of TANGL have the same aspect. But the structure of this part of the nucleolus has been investigated in particular

¹⁾ l. c. Taf. IIb fig. 30a.

²⁾ Archiv. 1882, p. 524, Fig. 162. Kern- u. Zellth. p. 7 and Tab. I, Fig. 2.

³⁾ STRASBURGER. Arch. 1882, p. 524. Kern- u. Zellth. p. 7. TANGL, l. c. p. 271. FLEMMING. l. c. Tab. IIb, fig. 30a, p. 159, 167 and 316.

⁴⁾ MEUNIER l. c. p. 351.

⁵⁾ l. c. p. 271.

⁶⁾ l. c. p. 347.

⁷⁾ l. c. Taf. IIb, fig. 30.

by MEUNIER. He crushed living filaments of Spirogyra, in a slightly alkaline solution of carmine, by a pressure upon the cover-glass and added after some minutes a few drops of diluted alcohol or some other fixing reagent. In the nucleoli thus treated, many of which had escaped from the nucleus and even from the cell, he found that a well-defined skein-structure became apparent. From all his observations he draws the conclusion, that this structure is common to all nucleoli of Spirogyra ¹⁾.

Respecting the chemical character of the nucleolus there is at present some confusion. ZACHARIAS ²⁾ and MEUNIER ³⁾ have both studied this question and though their investigations were conducted in nearly the same manner, they arrived at entirely opposite results. We can only wait for further experiments on this head.

Karyokinesis. This process takes place in cells, which have previously become somewhat longer than usual.

The nucleus at first increases in thickness and BEHRENS saw, that in living cells, in which karyokinesis is going on, the nucleolus seems to disappear after a certain time.

By observations made on cells of which the protoplasmic contents had been hardened by some reagent, several authors have come to the conclusion, that the nucleolus furnishes the material, either in part or entirely, of which the nuclear segments are formed.

STRASBURGER has propounded this view repeatedly ⁴⁾ though he has afterwards come to a very different one ⁵⁾. FLEMMING's ⁶⁾ observations led him to the same conclusion. Both authors do say but very little on the manner, in which the transition of chromatic matter into the segments takes place.

¹⁾ These and other observations lead MEUNIER to adopt Carnoy's peculiar opinion, who in his *Biologie cellulaire*, p. 236, regards the nucleolus of Spirogyra as an equivalent of the whole nucleus in other plants and therefore calls it „nucléole-noyau". For two reasons I will not follow him in this interpretation: 1. we do not find in the nucléole-noyau a smaller body, to be compared to the nucleolus and thus Carnoy's comparison is but a limping one. It is true that the existence of such a body is accepted by MACFARLANE (l.c.) and he calls it nucleolo-nucleus. But his observations have since been confirmed by nobody, neither have they been so by me. 2. I cannot see, how, on Carnoy's view, the greater body surrounding the nucléole-noyau and now universally termed nucleus, should be regarded.

²⁾ ZACHARIAS. Bot. Ztg. 1885, p. 273 and 1888, p. 90.

³⁾ l. c.

⁴⁾ STRASBURGER. Zellbild. u. Zellth. 3. Ed. p. 174, 184, 185; Arch. f. Mikr. Anat. Vol. 21, p. 524; Controv. p. 51, Separ.

⁵⁾ STRASBURGER. Ueb. Kern- u. Zellth. p. 190.

⁶⁾ l. c. p. 316.

Somewhat more explicit on this head are TANGL and MEUNIER. The first ¹⁾ figures and describes how the contents of the nucleolus are directly transformed into the segments, which are going to form the nuclear plate. MEUNIER ²⁾ states, that he saw the nucleolus lose its membrane at the beginning of the nuclear division, and that the skein, which forms the greater part of its contents, was divided into segments, but he does so under some reserve, remarking that it is extremely difficult to obtain perfect certainty about so small an object. Of all authors it is only ZACHARIAS ³⁾ who thinks it very improbable, that the nucleolus should furnish material for the nuclear segments and he does so in consequence of his chemical investigations.

The nuclear segments, once formed, lie all very accurately in an aequatorial plane, and the name of nuclear plate, formerly used for this stage of karyokinesis, seems here very appropriate indeed.

According to STRASBURGER ⁴⁾ it is very probable, that the number of segments constituting the nuclear plate is twelve. They split longitudinally and no doubt it is STRASBURGER's opinion ⁵⁾, that, when they separate, each of the longitudinal halves of the same segment withdraws in a different direction, so that never both take part in the formation of the same daughter-nucleus.

In the mean time the nuclear spindle has appeared, but as I have not made any observations on this process myself, I will refer the reader for this subject to the treatises cited above.

Between the two halves of the nuclear plate, when they have withdrawn towards the two sides, there now appears a certain amount of fluid, filling a cavity in the nuclear spindle. The amount of this fluid and therewith the size of the cavity increasing, the daughter-nuclei, for so we may now call the halves of the nuclear plate, are pushed farther away from each other. At the same time there occurs a bulging out of the walls of the cavity, already referred to, in an aequatorial direction, so that it seems as if a bladder, filled with fluid, extends between the daughter-nuclei. It is TANGL who has first described these phenomena ⁶⁾. He is of opinion, that the spindle-fibres extend at first through the fluid in the cavity, but

¹⁾ l. c. p. 277. figs. 13 and 14.

²⁾ l. c. p. 383.

³⁾ l. c. 1885, p. 280.

⁴⁾ Kern- u. Zellth. p. 11. Taf. I, fig. 18b.

⁵⁾ *ibid.* p. 16.

⁶⁾ l. c. p. 11, ss.

that afterwards they bend outward and disappear, by uniting to the wall of the cavity.

By STRASBURGER ¹⁾ some anterior observations of a similar description had already been made, but had been interpreted in a somewhat different manner. Afterwards ²⁾ however he has expressed his agreement in all essential points with TANGL's views.

It is generally known, that whilst these processes are going on in the nucleus, the formation of a cell-wall between the daughter-nuclei begins, and it first appears in the form of a diaphragm. This diaphragm becoming narrower and narrower, its inner margin meets at a certain moment with the bladder mentioned above and according to STRASBURGER ³⁾ and TANGL ⁴⁾ this takes place when the breadth of the diaphragm has become nearly one third of the cell-radius. As the diaphragm of cellwall grows further inward, the wall of the bladder between the daughter-nuclei is pushed back at the same rate, the bladder becoming narrower, till its cavity is wholly lost and there remains only a protoplasmic string, which, in the end, is cut through by the cellwall losing its central orifice. In STRASBURGER's and TANGL's drawings this phenomenon is repeatedly represented.

In a recent publication BEHRENS ⁵⁾ describes the karyokinesis of Spirogyra, as observed in the living filaments and he calls the cavity, filled with fluid, a vacuole with homogeneous contents. He took great pains in order to ascertain, whether this vacuole were possibly formed by the expansion of a very small vacuole previously existent. But he was unable to observe either this or any other mode in which the vacuole is generated.

Long before the vacuole between the halves of the nuclear plate is cut through by the cell wall, these halves have become real daughter-nuclei.

From TANGL's figures ⁶⁾ it might seem, that there is a direct transition from the halves of the nuclear-plate into the nucleoli of the daughter-nuclei.

STRASBURGER ⁷⁾ however has investigated this point somewhat

¹⁾ Zellbild u. Zellth. p. 176, ss. Taf. XI.

²⁾ Kern- u. Zellth. p. 18, ss.

³⁾ Kern- u. Zellth. p. 24.

⁴⁾ l. c. fig. 23.

⁵⁾ Bot. Ztg. 1890, p. 82, 85.

⁶⁾ l. c. fig. 21—24.

⁷⁾ Zellb. u. Zellth. p. 176. Arch. f. Mikr. Anat. Vol. 21, p. 526. Kern- u. Zellth. p. 23.

more particularly and comes in his description and figures to a different result. Several nucleoli are formed in or in contact with the mazes of the reticulum or skein, which constitutes a large part of the nuclear protoplasm in the daughter-nuclei. These nucleoli gradually fuse, till only one or two remain.

A few words may be added here concerning the relation between cellular and nuclear division.

Nuclear division, not followed by cell-division, has exceptionally been observed by several authors ¹⁾. A cell with two nuclei, having a diaphragm of cell-wall protruding but very little into the cell was observed by TANGEL ²⁾.

In normal cells the relation between nuclear and cell-division seems to be varying according to species. STRASBURGER ³⁾ mentions, that the diaphragm of cell-substance makes its appearance sometimes at the beginning of nuclear division, sometimes only after a nuclear plate has been formed. TANGEL ⁴⁾ states, that it occurs at the same time at which the formation of the nuclear plate begins.

The diaphragmatic cellwall, in growing towards the centre of the cell, of course pushes the peripheral layer of protoplasm, with its green spiral-bands, before it. But STRASBURGER ⁵⁾ has observed, that this phenomenon is of a somewhat more complicated nature, in as far as the spiral-bands do not adhere to the thin layer of hyaloplasm, by which they are lined on the outside and which fits closely to the straight cellwall and its diaphragm. They on the contrary bend from two points of the cellwall at some distance of the diaphragm towards the inner margin of this. (fig. 17 *e* and *h*.) By bringing about plasmolysis it is observed, that the outer plasmatical layer also leaves the cellwall and is contracted towards the spiral bands, coming again into contact with them ⁶⁾. At last the bands are cut through by the progress of the diaphragm. They are then drawn back and come again into contact with the cellwall, at first bending

¹⁾ cf. STRASBURGER. Zellb. u. Zellth. p. 183.

²⁾ l. c. p. 274.

³⁾ Zellb. u. Zellth. p. 360.

⁴⁾ l. c. p. 277.

⁵⁾ Zellb. u. Zellth. p. 181.

⁶⁾ From these observations the conclusion may be drawn, that the curious folds of the vacuole-wall (tonoplast), minutely described by de VRIES in Ber. d. Deutsch. Bot. Ges. VII, 1889, p. 19, as occurring often in abnormal conditions, are to be found in normal cells each time, that cell-division takes place and thus must be regarded as habitual phenomena.

at a right angle, so that they cover the peripheral part of the newly formed transverse wall. Afterwards they draw back still more, finally covering only the outer wall of the cell.

§ 1. METHOD.

With the exception of some observations, made on living filaments of *Spirogyra*, from a pond in the botanical garden at Groningen, the greater part of them was made on a thick *Spirogyra*-species, which I will describe afterwards. It was collected June 12th and 13th 1889 at 7^h 30' p. m. from a ditch in the vicinity of Utrecht. It was at that time of the evening full of cells in various stages of division. I was unable to cultivate it for a somewhat longer space of time in the laboratory.

But a large number of filaments was immediately put into FLEMING's mixture (chromic acid 0.75%, osmic acid 0.4%, acetic acid 4%). Here they remained for four days and after having been thoroughly washed out in pure water, they were put into a dialyser, containing water inside and 95% alcohol outside, this latter being renewed from time to time. Thus the transition from water to strong alcohol was effected without even the least degree of shrivelling. In this material some filaments were unfit for use, because the protoplasmic contents had contracted more or less. But in most of them there was no trace of any such change and in the cells of these threads all protoplasmic parts were in a really admirable condition. With another large species of *Spirogyra* some experiments were made by treating the threads with picric acid, dissolved in 50% alcohol, with picric-sulphuric acid (FOL), or a solution of 1% corrosive sublimate in water, but with all these fluids the results were very inferior to those with FLEMMING's mixture. Good results are to be had with 1% chromic acid, but material thus treated is much less adapted for the nuclear stain used by me.

The object of the succeeding manipulations is to imbed small pieces of the threads in paraffin, in such a manner, that series of consecutive sections may be made of them. I have had occasion to describe the general method, leading to this result, elsewhere¹⁾. But as the present material is somewhat difficult and necessitates the application of various peculiar precautions, I will shortly describe the whole process here, hoping thus to make it easy to control my observations.

¹⁾ Dodonaea, 1890, p. 325.

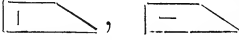
Some threads containing karyokinetic figures in the wished-for stages are selected and cut to pieces of from one to two millimetres in length, containing from ten to twenty cells. These pieces are transported with the aid of a small pipette into a glass box, containing a 6 % solution of horny, dry celloidin in a mixture of equal parts of aether and 90 % alcohol, which has been slightly stained with gentiana-violet, in order to facilitate its being distinguished in further operations. A certain amount of water in this solution is necessary; no absolute alcohol should be used.

Here they remain for some minutes and are then transported with a drop of the celloidin-solution, by means of a pipette, to a glass slide. The drop of fluid flows out and forms a thin layer, in the midst of which the thread lies. In a few moments there appears a film on the surface and then the slide is put into a glass vessel, filled with 95 or 96% alcohol. Here the celloidin layer soon becomes of a suitable consistency and, using a razor in the manner of a chisel, it is cut into pieces of nearly a square centimetre, each containing a piece of Spirogyra-thread. These pieces can be easily removed from the glass and are put again into 96% alcohol, which should this time be stained with gentiana-violet or some other dye somewhat more intensely, e. g. till it loses its transparency. Here the celloidin-pieces remain for $1\frac{1}{2}$ hour or more, becoming of a homogeneous consistency and a very dark colour.

Now the alcohol should be replaced by oil of marjoram (origanum), in order to make a transportation into paraffin possible. Here some caution is necessary, for the celloidin has not penetrated into the cells and the transition to oil of marjoram is very likely to cause a large degree of shrivelling. Sometimes, especially if the threads contain only cells with resting nuclei, it is sufficient, previous to bringing the celloidin-pieces in pure oil of marjoram, to let them lie for some time in a mixture of 6 parts of this oil to 1 part of 96% alcohol. But in most cases, and especially if cells in stages of division be present, this precautionary measure is wholly insufficient. The only really safe mode is to bring at the bottom of a small glass cylinder some amount of the mixture referred to above (not pure oil of marjoram) and upon it, by means of a fine pipette, and in such a manner that both fluids do not mix, a thick layer of the coloured alcohol. The celloidin-pieces will, without losing their colour, quickly sink down to the boundary plane between the fluids and then will very gradually sink to the bottom. After a lapse of one or two hours they may be transported without injury into pure oil of marjoram. If the Spirogyra-threads, contained in the celloidin-pieces, be

now examined under the microscope, it will be seen, that there is not even the slightest sign of shrivelling and further, that those cells, which have been opened, when the threads were cut into pieces, are now filled with celloidin. This becomes manifest by their having the same tinge of colour as the rest of the piece of celloidin, whilst all intact cells, being filled with uncoloured oil of marjoram are of a much lighter tinge. For the rest all parts of the cells are to be distinguished admirably well, though they are stained only in a very feeble measure.

There are now made some preliminary sketches of the piece of thread in hand, first of the whole, magnified some 50 diameters, then of each nucleus separately, under a somewhat higher power e. g. 250 diameters. I found that these sketches are essential, if afterwards the results should be judged of with precision.

The piece of celloidin is then carefully cut with a razor into one of the shapes represented here:  making it easy to know without microscopical examination at which side the Spirogyra-thread is to be found and the precise direction in which the sections are to be made.

The transport of these pieces into paraffin is again a tedious process, from the great tendency to shrivelling. By experiments I learned, that it is necessary to convey them successively through solutions of 15, 30, 45, 60, 75 and 90% paraffin in oil of marjoram. These are kept in little, well-stoppered glass vessels, in small quantities, and thus the celloidin-pieces may be easily picked up with a spatula. A stay of 10 or 15 minutes in each solution is sufficient. If the pieces thus treated, be examined at last under the microscope, in a medium of pure, molten paraffin, it will be seen, that there has not taken place even the slightest change in the cells. Finally the pieces are imbedded in the usual manner in a rectangular block of paraffin over which cold water is poured, as soon as it begins to cool and a thin film appears at its surface.

When the block of paraffin has been clipped with a razor to make it fit for the microtome, the deeply coloured piece of celloidin will shine through and it will be a very easy matter to adjust it on the microtome in such a position, that its sharp edge, indicating the direction of the sections to be made, is parallel to the edge of the razor. In this manner a really surprising exactness may be reached, so that it is by no means difficult, to make a series of longitudinal sections of a Spirogyra thread, all being parallel to its axis.

The greater part of the sections described in this paper was made

with de GROOT's microtome ¹⁾ and most of them had a thickness of 5, some of 10 μ .

The ribbon of sections obtained is laid on a glass slide and examined under a low power of the microscope, in order to find out, which sections contain parts of the Spirogyra-thread. It now again becomes evident, that only those cells, which have been opened, contain celloidin, their contents being quite transparent as well as the surrounding celloidin. But the intact cells contain only paraffin, showing the same contorted structure, which is to be observed in the parts of the sections surrounding the celloidin. This proves at the same time the fact, that the molten paraffin has penetrated through the coating of celloidin into the cells. In a few cases small empty cavities are to be observed in some cells, and these cells are of course worthless. I do not know the cause of this defect and have not been able to eliminate it in all cases.

That part of the ribbon, which contains the sections, is now glued to a slide, with albumen, in the usual manner. Have the sections become somewhat wrinkled, this can be previously remedied by floating the ribbon for a few moments on the surface of tepid water, but in most cases this ceremony can be dispensed with.

The slides are heated and then put into turpentine, in order to solve the paraffin, then into 95% alcohol for removing the turpentine. Two glasses filled with each fluid are consecutively passed through. In turpentine the slides should remain for as short a time as possible e. g. not for longer than one hour, because this reagent has, after a prolonged stay, sometimes only after many days, a very detrimental effect. At last the whole of the protoplasm becomes disorganized, the chromatic substances are lost and the cellwalls become swollen, showing a quantity of very fine strata. For staining the sections I used always a very dilute watery solution of Gentiana-violet R. from TROMMSDORFF (one part of a concentrated solution in 95% alcohol to thousand parts of water). The slides remain in this fluid from one to three hours, at a temperature of 60° C. They are then dipped for a single moment into absolute alcohol with $\frac{1}{10}$ % of hydrochloric acid — for the same space of time into alcohol, to which a single drop of caustic ammonia has been added and finally they are washed for a very short time in neutral absolute alcohol. Then some drops of oil of cloves are added to the sections, which are now ready for examination. They were in most cases mounted immediately afterwards in Canada-balsam, or a solution

¹⁾ Zeitschr. f. wiss. Mikr. IV, 1887, p. 145.

of dammar or colophony in turpentine. According to the time passed in the staining solution, different results are obtained and I have found this fact largely profitable for bringing to light different structures. It is from this cause, that on the plates illustrating this paper, the figures are of very various intensity. I will return to this subject in discussing the results of my investigations.

If it be wished to remove the celloidin from the sections before staining, this can be brought about by bringing the slide into a mixture of aether and alcohol. But it is hardly worth while, because in oil of cloves almost all celloidin is quickly dissolved and should any traces of it remain, I have never found, that they were in the least degree inconvenient; on the contrary these slightly-stained patches facilitate the finding of the diminutive sections under the microscope. This is often a tedious task and it is almost always necessary to mark in some manner the spots on the slide, where nuclei are to be found. In the case of transverse sections, it will be advisable to give a number to each nucleus, corresponding to a number in the preliminary sketch.

It is clear, that if the method described here, gave always good results, it would be of much importance for the study of microscopical organisms. But this is not the case, at all events not with objects such as *Spirogyra*, the cells of which possess large vacuoles. I have told, that celloidin does not penetrate into intact cells and this is the cause of a strong propensity to shrivelling. This obstacle however, as we have seen, may be removed by the precautions, mentioned above. But when the sections are made, the same cause will produce evil effects, which I have till now not been able to eliminate.

When the edge of the razor is passing from paraffin into the celloidin, which has been soaked with paraffin, there is no difficulty, but when, on entering the imbedded thread, the reverse takes place the softer paraffin-mass will be loosened from the neighbouring celloidin and somewhat compressed. Thus some parts of the cells will be crushed and will get out of place. The nuclei, lying in the centre of the cells, often remain in their places and this circumstance has made my investigations possible. But in most cases all elegance of the preparations is lost and in some a total failure is the result.

With those cells, which were opened and filled with celloidin, it is quite the reverse. All parts remain intact and fully preserve their original places and in a few instances, in which such cells had not suffered quite too much, by having been cut through, magnificent specimens were obtained.

For these cells moreover, shrivelling is not to be feared, even if they be transported at once from alcohol into oil of marjoram or from this into paraffin. If means could be found to introduce the celloidin into the cells all tedious operations of the method could also be dispensed with¹⁾.

With the help of this method, such as it is, the following observations were made.

§ 2. OBSERVATIONS.

In the first place I will give here a short description of the *Spirogyra*-species on which my observations were made, and fig. 19, representing a transverse section of a whole cell, will illustrate it.

The average thickness of the filaments in alcohol, obtained from 22 measurements is $135\ \mu$ (minim. 120, maxim. $150\ \mu$). The average length of the cells is $100\ \mu$ (35 measurements, min. 80, max. $150\ \mu$), but if they are in a state of division it is somewhat greater, viz. $158\ \mu$ (18 measurements, min. 120, max. $190\ \mu$). Thus it appears, that the average difference in length between resting and dividing cells is not inconsiderable, but that in particular cases the difference may be very small or even inverse.

The transverse cellwalls of this species show no annular folds. The cellwall is everywhere thin and has no slimy coating. The number of spiral-bands is very variable in different individuals. In fig. 19 there appear 17. I often counted them and found also the numbers 12, 13, 14, sometimes 23 or 25. They are inclined at an angle of 50° towards the axis of the filament.

¹⁾ It is obvious, that I have made some trials, in order to promote the introduction of celloidin into the cells, but till now without success. For those, who should wish to improve the method, I will mention, that the following experiments were made without result:

The celloidin was dissolved in glacial acetic acid, absolute alcohol, methylic alcohol, acetone, acetic ether and oil of cloves. In order to seduce the cellwall to the admission of celloidin, filaments were previously treated with $\frac{1}{4}\%$ chromic acid at 60°C ., 20% chromic acid, an alcoholic solution of caustic potash, picric-acid, chloride of zinc, nitric acid. No better results were obtained with *Spirogyra*-threads, treated when living, with the reagents mentioned at p. 9.

A stay of 43 days in the solution of celloidin had no effect.

It was tried to open the cells, by alternately freezing and thawing the threads four or six times and by means of a sharp needle under the microscope.

Instead of celloidin the use of photoxylin and gelatinous silica was tried.

Some experiments with the thallus of *Lunularia vulgaris* showed, that here the system of intercellular spaces is easily filled with celloidin, but that it does not penetrate into the cells.

The pyrenoids were always distinctly stained, in many cases much more distinctly than in fig. 19, representing a specimen, which had been dyed for one hour only. Often they were stained almost as intensely as the nucleolus, (Fig. 17 *e* and *h*). FLEMMING ¹⁾ already made the same observation. By adding an alcoholic solution of iodine and afterwards glycerine, the starch granules, arranged circularly around the pyrenoids are coloured blue and appear with much distinctness. The protoplasmic threads, by which the nucleus is suspended in the middle of the cell, I very often saw terminating in the amyllum-bodies, never elsewhere (fig. 19, 17 *e* and *h*).

Filaments in the act of conjugation I have found in abundance. Not all cells of a filament take part in this process; by a great number no conjugating processes are produced. These cells become very transparent, they contain no drops of oil, their chlorophyll-bands become very narrow, their amyllum-bodies very small. The copulating cells on the contrary show large amyllum-bodies, filled with starch, and broad chlorophyll-bands, whilst a very great number of oil drops is stained black by the osmic acid of FLEMMING's mixture. There is some difference between the cells of the feminine thread, which swell out towards the masculine one, and those of the masculine thread, retaining their cylindrical shape. Though so many copulating filaments were seen, I observed not a single zygospore: in all cases the protoplasmic contents still retaining their place. As moreover my material was collected for two subsequent days at the very same locality, it is probable, that the cells in this species remain copulating for a somewhat long time, before the zygospores are formed. STRASBURGER has described the same phenomenon ²⁾.

Of the so called attractive spheres, recently described by DE WILDEMANN ³⁾ for *Spirogyra jugalis* and *nitida*, I have not been able to see anything, and this most probably should be attributed to my methods of staining and mounting.

It is a well known, but lamentable fact, that the species of *Spirogyra* in many cases present unconquerable obstacles to their identification ⁴⁾, because our systematical knowledge of this genus is a very defective one. This is partly caused by an unhappy choice of specific characters. But the defect will never be thoroughly

¹⁾ l. c. p. 160.

²⁾ Zellb. u. Zellth. p. 171, Kern. u. Zellth. p. 5.

³⁾ Sur les sphères attractives dans quelques cellules végétales. Bull. Ac. Roy. de Belg. 1891. p. 594.

⁴⁾ STRASBURGER Zellb. u. Zellth. p. 171.

remedied, until it becomes possible to cultivate these plants with some degree of certainty and any one who has tried it, will know, that this very often is by no means so easy, as the unsuspecting student is sometimes led to believe.

Thus it is not to be wondered at, that in many cases, in which nuclear and cellular division of these plants were investigated, the names of the species, on which the observations were made, have remained uncertain or wholly unknown ¹⁾. Of the species, recorded here, the following is all I can say. By consulting some of the best systematical works on Algae, I found that only the description of *Spirogyra crassa* Ktz corresponds, if not wholly, at all events in most essential parts with the characters I observed in my plant. These agree also very well with the description given by STRASBURGER ²⁾ of *Spirogyra crassa* Ktz.

But they agree as well with the description, given afterwards by the same author of another species, which he distinguishes from *S. crassa* Ktz. as *S. polytaeniata* (STRASBURGER?) ³⁾. Moreover I found myself at Groningen a *Spirogyra*-species, being, according to the systematical works, no other than *S. crassa* Ktz., but being at the same time without doubt a species different from that, on which my observations were made, as for instance its nuclei were not disciform but globular.

Under these restrictions I will call the species, I used in my investigations, *Spirogyra crassa* Ktz.

I will now begin with a description of the nucleus in its resting state. It is disciform and shows in almost all cases a very distinct membrane (fig. 20, 21, 23, 25). The number of nucleoli varies from one (fig. 19, 23, etc.) to two (fig. 20, 21, 28).

The shape of the nucleus, as shown in a longitudinal section is not always the same. Sometimes it is lenticular and thick, so that the nucleoli are thinner than the nucleus (fig. 25), but sometimes also it is flatter and thinner, so that the nucleoli are thicker than the nucleus and protuberate. A section of such a nucleus is drawn in fig. 20, of an intermediate form in fig. 23.

In filaments composed of short cells, none of which are dividing, only thin nuclei are to be observed. But in filaments, in which many cells are in the act of dividing, they are seldom met with, and if so,

¹⁾ STRASBURGER Zellb. u. Zellth. p. 171; FLEMMING l. c. p. 315; BEHRENS l. c. p. 81; MEUNIER l. c. p. 340.

²⁾ Zellb. u. Zellth. p. 171 and 186.

³⁾ Kern- u. Zellth. p. 4.

only in cells which are particularly short. Most resting nuclei in such threads are thick ones. The same can be said of the nuclei in cells, which have just divided, as is shown by the protoplasmic string, extending from one nucleus to the other. From these facts I conclude, that thin nuclei are the true resting ones, the thick nuclei being near to the dividing condition, either before or after it.

Respecting the distribution of the chromatic substances in the resting nucleus, it may be said, that only the nucleoli retain gentiana-violet with obstinacy; from all other parts it is extracted without difficulty. This is true concerning intensely-stained nuclei (for 3 hours, figs. 20, 23, 25) as well as concerning feebly-stained (for 1 hour, fig. 21) ones.

The nuclear protoplasm shows a finely-reticulate structure (fig. 21), but in the transverse sections of nuclei not much of this can be seen. It is more difficult to answer the question: which is the structure of the nucleolus?

As will be seen from the figures, three different cases occur. Some nucleoli present themselves as a homogeneous, black mass (figs. 23, 25 and the lower nucleolus of fig. 20). Figs. 20 and 21 show a more or less evident skein-structure and in fig. 26 an isolated nucleolus is seen, in which the presence of vacuoles seems to be indicated.

Homogeneous nucleoli are only to be found in specimens, stained for three hours and even then they are comparatively scarce. The skein-structure is often quite evident and is to be found in very many cases. With regard to the occurrence of vacuoles, it must be observed, that certainty is here more difficult to obtain. But in many cases, in which thin slices of nucleoli had been cut off, and in some sections of 2 μ . thickness, made on the purpose, I saw them with absolute certainty and I must observe, that fig. 26, being a whole nucleolus, does not give an adequate idea of the distinctness with which I often saw them. They occurred in my sections much oftener than homogeneous nucleoli, but not so often as the skein-structure.

I now proceed to the inspection of nuclei, occurring in elongated cells and showing by their swollen and peculiar appearance, at the first glance, that the process of division has begun. Transverse sections of the filaments are here necessary, for thus the internal changes can best be seen.

I first direct the reader's attention to figs. 22 and 30, of which figs. 1 and 2 are the preliminary sketches. The protoplasm of both exhibits a fine network, essentially the same as in fig. 21. But moreover there is a slender, thread-like structure; of which it was impossible to represent the whole, for fear of embroiling the figures.

As far as could be judged of, the parts of it, drawn in the figures, were all united to each other and combined to form a single coil, issuing from the pointed end of the now slightly pear-shaped nucleolus. It will not be superfluous to remark, that the point of the nucleolus and the beginning of the thread were seen exactly at the same level. This thread itself is formed by a feebly-stained stroma, in which a regular row of more intensely-stained particles can be observed.

In both figures the nucleoli show somewhat brighter spots, as if there were vacuoles, and this particular is much more obvious in fig. 31 (preliminary sketch in fig. 3), representing the same stage of a somewhat less intensely-stained nucleolus. In this nucleolus a skein-structure is also to be seen.

Fig. 29 again represents the same stage of karyokinesis, but much more feebly-stained and here the skein-structure of the nucleolus is still more apparent. About this figure it should be further remarked, that the pointed end of the nucleolus was at the same level with the right hand loop, seeming to issue from it, whilst that to the left was at a somewhat lower level.

A feebly-stained nucleolus at a somewhat later stage is seen in fig. 28 *a* and *b*. This was a very thick nucleus, so that four sections were made from it, of which however only the two central ones are figured, the external ones being only slices of nuclear protoplasm. In these figures no parts were omitted, so that it is evident, that there is here not a single thread, but a certain number of segments containing chromatin, the nucleoli also containing some stained threads.

The observation of this stage is completed by fig. 27, corresponding to the preliminary sketch fig. 4. Only one section of two, containing segments, has been drawn here and the specimen was deeply-stained, viz. for three hours. The nucleolus notwithstanding is less stained than those of figs. 22 and 30, but it shows with much clearness the vacuoles, already noted in these figures and particularly in fig. 31. In the nuclear plasm are found twelve or thirteen segments, consisting of a faintly-stained intermediate substance, containing some sharply-defined chromatin-bodies. Here the network of nuclear plasm, which was missed in the feebly-stained specimens, appears again.

I now pass to Pl. II, where fig. 32 *a* and *b* show the two central ones of four sections from a nucleus, represented in fig. 5. It has already lost its rounded form and there are no traces of a nucleolus. This specimen again was feebly-coloured and shows twelve segments, which seem to consist wholly of chromatic substance, the achromatic, intermediate substance being no longer visible. The tinge of the nuclear

plasm is relatively somewhat darker than at the foregoing stages.

At both stages, represented in figs. 27 and 32, the segments lie by no means in an aequatorial plane, as is evident from the facts, that they are divided over two sections and that some of them had to be foreshortened in the drawing.

Through these transitional stages we are led unperceptibly to the nuclear plate. All segments here lying at the same level, beautiful specimens easily are to be got, as is shown by the figures 33, 35 and 37, of which the figures 6, 8 and 7 respectively represent the preliminary sketches.

In figs. 33 and 37 are drawn the central sections of very thick nuclei, one with twelve, the other with thirteen segments, none of these being quite straight. Thirteen segments were observed but once, twelve often, so that this is the normal number. From the preliminary sketches it goes forth, that in these cases no longitudinal splitting of the threads had as yet taken place. Fig. 37 again clearly exhibits the reticulum in the nuclear plasm, already observed in the resting nucleus and some dividing stages.

The nuclear membrane appears still with great distinctness in fig. 33, but in fig. 37 it is lost, so that this last figure corresponds to a somewhat later stage, a conclusion which is corroborated by the fact, that the angular transitional form of the nucleus in figs. 5 and 6 is changed in fig. 7 into a more rounded one, which will remain in subsequent stages. The figures 33 and 37 both represent intensely-stained nuclei.

In fig. 35 we meet with a nuclear plate, in which longitudinal splitting has already taken place, as can be seen in the preliminary sketch, fig. 8. The difference between this nucleus and those of figs. 33 and 37 is but small; still it may be said, that the segments are somewhat thinner.

I will call the reader's attention also towards a somewhat more advanced stage, of which a preliminary sketch is drawn in fig. 9. The splitting of the nuclear plate and the separating of its halves are quite apparent. Transverse sections should have been made of this nucleus, but the result was, that they became somewhat oblique and thus were unfit for being drawn. But in this specimen it was very evident, that each segment corresponded with an exactly parallel one at a small distance.

I now pass to the phenomena, occurring in the nuclear spindle, between the two halves of the nuclear plate, destined to become daughter-nuclei. Here it will be necessary to have recourse to series of longitudinal sections.

In the stages of karyokinesis treated of till now, I have diligently sought for some vestiges of vacuoles in the vicinity of the nuclear plate and between its halves, but always in vain.

In fig. 36 *a* and *b* two longitudinal sections have been drawn from a nucleus, with nuclear plates already widely separated. Between them the spindle-fibres are very clear and at two spots these are somewhat thicker, but of a vacuole nothing is to be seen with certainty.

Fig. 34 illustrates a later stage, intensely-coloured, so that the spindle-fibres cannot be distinguished, but here the presence of a vacuole is evident. It does by no means occupy the centre of the spindle. This is the earliest stage, at which I have observed a cavity.

A complete series of longitudinal sections of a subsequent stage is figured in fig. 15 *a-f*. A large vacuole, here also occupying a lateral position, is to be seen, and moreover some smaller ones appear. Repeatedly I had occasion to observe the fact, that in this stage the occurrence of three or four vacuoles is the rule.

It is illustrated by fig. 38, being the fourth of a series of six consecutive, transverse sections from the nucleus, delineated in fig. 10. The same can be said of fig. 11. Of this nucleus seven transverse sections were obtained and the fourth one of these showed essentially the same aspect as fig. 38, though the protoplasmic walls had become thinner and the vacuoles larger. In the same measure as the nuclear spindle bulges out more and more, one of the vacuoles becomes more prominent, as is shown by fig. 18 *a-d*, representing four sections from a stage, in which the diaphragm of the new cell-wall has already reached the extending nuclear spindle. But the protoplasmic layer, limiting this vacuole, is of considerable thickness.

Four consecutive transverse sections, or parts of them, from a more advanced stage of karyokinesis are to be found in figs. 42 *a-d*. The preliminary sketch of it is fig. 13 and it will be seen, that the vacuole was here of a somewhat irregular shape. The direction of the sections was slightly oblique. Eight sections were obtained and of these fig. 42 *a* is a part of the second, 42 *d* of the fifth one, reckoning from the right hand side of fig. 13. Thus some parts of the protoplasmic layer, surrounding the vacuole, are seen from above and it appears, that they are very finely-striated, the lines being somewhat thicker at the aequator.

In fig. 17 *a-i* we see a series of longitudinal sections from a stage, in which the cell-wall-diaphragm has protruded a little further. Here again only a single, large vacuole is to be seen, diminished

in aequatorial diameter, as compared to fig. 18, and separated by a thin layer of protoplasm from the cell-sap. It is only in fig. 17e that perhaps a small vacuole besides the large one appears at the right hand side.

The succeeding stages show always a single vacuole, generally of a regular shape. Fig. 24 e. g. represents the middle section of fig. 14.

A series of sections through a still more advanced stage is seen in fig. 16 *a-h*. Here the daughter-nuclei are united together by a protoplasmic filament, still showing its vacuole in the vicinity of one nucleus, whilst it has almost disappeared at the other end.

Lastly we see in figs. 40 and 41 two nearly median sections through nuclei in the last stage of karyokinesis. In the last-named figure the remnant of the nuclear spindle has been cut through by the new cell-wall, though it still exhibits its central vacuole, divided into two parts, and even traces of a couple of smaller ones.

I now return to the nuclear plate, which we have left at the moment, that the twelve segments had splitted longitudinally and had begun to separate.

Comparing the figs. 36 *a* and *b* to 34 and 15 it is obvious, that the segments fuse together. Thus in longitudinal sections the appearance of separate fragments is modified into that of a homogeneous plate. Still this is only in appearance, as a glance at fig. 39 will show. This represents one of the daughter-nuclei of fig. 12, seen from above. There has been formed a somewhat complex skein, having apertures between its loops.

When the vacuole between the daughter-nuclei has expanded, as in fig. 18, the aspect is changed and the nuclear plate has made place to a daughter-nucleus, showing one or two nucleoli. These however at first by no means contain all chromatic substance and this fact is illustrated by the figs. 16 and 42, especially the last one. Here is easily observed a structure, often recalling to memory that of the opening stages of karyokinesis, viz. one or two nucleoli and a large number of smaller chromatic fragments, generally distributed in rows through a slightly-coloured, intermediate substance. However, this substance does not present itself in the shape of a single filament as in figs. 22, 30 and 31, but it obviously is a network. Finally the smaller, chromatic fragments disappear, one or two nucleoli remain and the nucleus returns to its resting state. After this description of my observations on karyokinesis, I will record in a few words those on the relation between this phenomenon and cell-division.

The first traces of a transverse cell-wall in the shape of a diaphragm, I once observed in a cell, having a nucleus in the stage of fig. 32, the segments being just formed. But it was an exceptional case and in all others, in which this or an anterior stage of karyokinesis appeared, the formation of a cell-wall had not begun. But in all cases, in which I saw a nuclear plate, though still without signs of longitudinal splitting, the cell-wall had just made its appearance, and so it was in the cells belonging to figs. 33 and 37. In that belonging to fig. 35 the diaphragm had penetrated already a little further, but still for only $\frac{1}{11}$ part of the cell-radius.

The stage of the undivided nuclear plate consequently corresponds to the first beginning of cell-division.

The process can however be delayed sometimes. Thus the cell containing the nucleus, which is delineated in fig. 9, showed no traces of division, though the halves of the nuclear plate had already separated. I even found two cells, containing nuclei in the stage of fig. 36, in which the formation of a cellwall had not begun.

Lastly I will mention the case of a cell, in which karyokinesis had nearly come to an end, for it contained two nuclei, only united together by a protoplasmic string without a vacuole. Still the diaphragm had protruded only for $\frac{1}{4}$ part of the cell-radius. But these nuclei were not sound ones. They were stained irregularly in all their parts, and no nucleoli were seen in them.

In transverse sections the cell-walls dividing the nuclei are to be seen very well, as they are stained not intensely but still distinctly, which is also shown in fig. 19. But they seldom are quite plane, almost always bulging out a little towards one of the two cells.

From this cause such a cell-wall is generally divided over two sections, one containing a circular, central part, the other a diaphragm, into the aperture of which the central part nicely fits. Of the chlorophyll-bands nothing is seen on these cellwalls. If however there be no complete partition, but only a diaphragm of cell-substance in a dividing cell, it is to be observed, that these bands protrude upon the diaphragm even as far as one half of the cell-radius. They are placed obliquely, calling to the mind a partly-closed iris-diaphragm, with this exception that the bands leave a more or less considerable distance between them.

§ 3. RESULTS.

In judging of the conclusions to be drawn from my observations, it should by all means be kept in mind, that they were made from hardened material.

The principal new facts, which were brought to light, are moreover of such a nature, that probably for some time to come, it will not be possible to see anything of them in the living cell.

Still I do believe, that there is a large degree of probability, that these phenomena are recorded essentially as they take place in living nuclei. I here observe, that the new facts, promulgated in this treatise, chiefly relate to the observation of transitional stages between some well-known stages of karyokinesis, easily seen in living cells, as well in the case of *Spirogyra* as in other ones. These principal stages are found to exist in my specimens with the utmost accuracy and the same may be said of all other parts of the cell, as well as of the nucleus. Thus, I think, there can be no objection to view the transitional stages also as genuine, unless there be wighty reasons, why in these cases the methods used have not been efficient. Such reasons however, as far as I can see, do not exist. Therefore I do not hesitate from my observations to draw conclusions about changes of form and movements which have taken place. But I will by no means, as some authors have done, extend my speculations towards the forces causing such changes.

Another observation, which should precede the discussion of the results, concerns the nature of the staining-process, used by me. *Gentiana-violet* is one of the best nuclear dyes and is not easily surpassed in demonstrating those elements of the nucleus, called chromatic and the interesting changes they undergo during the process of karyokinesis. But at the same time it should be kept in mind, that some parts of the cell, having no relation with the chromatic nuclear elements, are quite as tenacious and even more so of this dye.

FLEMMING's observation¹⁾, that the pyrenoids seize hold of aniline dyes with almost the same tenacity as the nucleolus, was confirmed by me and in a less measure the same may be said of the cell-wall. (fig. 19). It is moreover a curious fact, that the spiral-bands of spiral-vessels in the most different plants take hold of *gentiana-violet* with a truly wonderful force. In specimens made with the object of studying the nuclei, but extracted for too long a space with acid alcohol, so that the nuclear stain had vanished, the spiral-bands remained very intensely violet. I also possess specimens, containing sections of the ovules of *Scilla sibirica*, etc., made four years ago and stained with *gentiana-violet*. When first made, the nuclei were beautifully coloured, but now the colour has vanished from

¹⁾ l. c. p. 160. Taf. IV^a, fig. 47.

all parts with the exception of the spiral-bands, these retaining their original brightness. I have no doubt, that for the study of the distribution and the development of spiral-vessels this and similar dyes can be of much importance.

Knowing such facts, it will be necessary to reckon with them, in judging of the results of these researches, but I am confident that the sequel will show, that this has been done.

A second observation to be made about the staining is this, that it was effected by what several authors not inappropriately call the regressive method. It should be kept in mind, that by this process all parts of the cell and even the albumen, by which the specimen is fastened to the slide, are at first very intensely stained and that afterwards, by means of slightly-acidulated alcohol, the stain is extracted from certain parts more than from others.

With regard to the result, thus to be obtained, the space of time, during which the specimen remains in the staining solution, is a very important factor. If this space be very long, for instance some hours and if at the same time the extraction be moderate, the result can be, that all parts of the cell: cell-wall, protoplasm, nucleus, etc.. are stained with nearly the same intensity. And by modifying the treatment in these particulars all gradations may be obtained, till the almost exclusive staining of the nucleoli.

The knowledge of these facts wholly excludes the use of such staining methods as a kind of chemical reagents, by which certain substances, to be called chromatic, are always brought to light with the exclusion of others. But on the other hand, this method has many advantages for making visible various parts, differing in their tenacity of some particular staining reagent. By staining for a long time, those parts, which have only a small attraction for the dye, will also become visible and thereby the more chromatic elements will be covered more or less. By staining for a short time the first-named elements will remain invisible, the so-called chromatic ones becoming more clearly distinguishable in proportion.

From these considerations we will largely profit in drawing conclusions from my observations.

Concerning the nuclear plasm I was able to confirm the observation, that it is surrounded in the resting nucleus by a distinct membrane, remaining visible during karyokinesis even till the stage of the nuclear plate (fig. 33), but disappearing very soon afterwards (figs. 35, 37). In the daughter-nuclei, delineated in figs. 15 and 39, it has not yet reappeared, but in fig. 42 this is the case.

In the nuclear plasm itself we find through several stages of karyo-

kinesis, until the nuclear plate appears, a fine reticulum, which however is only visible in intensely-stained specimens, as a comparison of figs. 21, 22, 30, 31, 27 and 37 with figs. 28, 32, 33 and 35 will clearly show. The reticulum delineated by FLEMMING¹⁾ is much coarser than that observed by me, which agrees better with that represented by MEUNIER in several of his figures. In stages, subsequent to that of the nuclear plate, this structure disappears at the same time with the nuclear membrane and the fusion of nuclear and cellular protoplasm.

In young daughter-nuclei I have not been able to retrace the existence of this reticulum, at least it seems doubtful to me, whether the coarser reticulum, drawn in fig. 42 *a* and *b* be the same thing as the finer one, represented in the figures of the mother-nucleus.

Be this as it may, it is very certain, that in the cytoplasm of the resting nucleus no appreciable amount occurs of substances, comparable in their affinity to gentiana-violet to those constituting afterwards the so-called chromatic figure. On this head I can only corroborate the testimony of the authors cited above.

I now proceed to the structure of the nucleolus, the consideration of which will lead us to that of the chromatic figure. As it is no easy task to judge of this structure, I have not only made the observations already described, but I also repeated some of those of Meunier, bearing on this subject²⁾. I made use for this purpose of a thick Spirogyra-species, already mentioned at p. 16. For reasons enumerated there, I will let it unnamed.

Filaments of this plant were brought into a drop of a watery solution of ammonio-acetic carmine (GRÜBLER's carminsaures Ammoniak, Hoyer), to which a trace of caustic ammonia had been added. If too much of this substance should be present, no staining will take place. These threads were then crushed between slide and cover-glass and by this process many nucleoli became wholly free and were seen floating through the fluid, others remaining in their places, but all stained. After the lapse of some minutes, 50 or 96 % alcohol was caused to replace the original medium and sometimes a drop of glycerine was put at the edge of the cover-glass, the specimens thus remaining longer fit for observation, but otherwise not becoming altered. The results obtained, agree in the main with those of Meunier, though I was by no means able, to get specimens, comparable in distinctness to his figs. 33—37.

¹⁾ l. c. Taf. II^b. fig. 30^a.

²⁾ l. c. p. 370.

On 116 floating nucleoli, which were observed by me, I found 29 of which the skein-structure was really apparent, the loops being more or less free from each other. In these cases it was also apparent, that this skein contained the red stain, by which all these nucleoli were distinguished. In 64 nucleoli the same structure was less distinct, but its presence still probable in the highest measure. In 21 I was unable to make out any structure and in 2 only, I observed more transparent, circular spots, reminding of vacuoles.

The reader will please to remember, that in the specimens, recorded in a former paragraph, I saw a few nucleoli homogeneously stained, many exhibiting a skein-structure and also a large number, containing vacuoles.

The homogeneous appearance of some nucleoli can be easily explained by their having been very intensely stained (see. p. 17) and in connection with the fact of their being few in number, there is no reason why, even in these cases, all structure should be wanting.

The presence of vacuoles was seen in many instances with as much certainty as is to be obtained, where the application of plasmolysis is excluded. One of FLEMMING's figures ¹⁾ also shows the same. I further direct the reader's attention to the nucleoli, represented in figs. 22 and 30, and especially in figs. 31 and 27.

These figures show, that intensely-stained nucleoli, which have lost their chromatic substances totally or in part, show vacuoles very clearly. Slightly-stained specimens, such as are seen in figs 29 and 28 exhibit nothing of this kind.

From all these facts I draw the conclusion, that in the resting nucleolus vacuoles are often present; it even appears by no means improbable, that this is always the case, but that sometimes their diminutiveness, combined to a feeble staining is the cause of their not being seen.

In accordance with this view is the fact, that vacuoles in normal nucleoli of plants and animals are of very frequent occurrence, so much so, that FLEMMING ²⁾ from the facts known to him, regards the universal occurrence of vacuoles in all nucleoli, not indeed as certain, but still as by no means improbable.

MEUNIER did not see them, neither did I, when I repeated his experiments, excepting a few instances. But this seems not incompatible with the foregoing view. For in these experiments

¹⁾ l. c. Taf. II^b. fig. 30^a.

²⁾ l. c. p. 151.

the living nucleoli were brought from their natural medium into a fluid fit to kill and to stain them, but not fit to arrest living protoplasm in its original form. Thus it is not to be wondered at, that the extremely delicate vacuoles disappeared by such a treatment.

Moreover MEUNIER, though in general regarding the appearance of vacuoles in nucleoli as a pathological phenomenon, has still observed them in normal nuclei also ¹⁾.

Concerning the skein-structure of the nucleolus it should be borne in mind, that it was not only often observed in the resting nucleus (figs. 20 and 21), but that it always appeared in the first stages of karyokinesis, provided that the staining was not too intense (figs. 31, 29, 28). In those numerous cases, in which vacuoles were observed, the coexistence of a skein-structure was not at all excluded (fig. 26) and adding to this Meunier's experiments with carmine, repeated by me with nearly the same results, there is ample reason to conclude, that a skein-structure generally occurs in these nucleoli. It follows further from all observations, that the thread, here referred to, contains the chromatic substances of the nucleus.

Resuming the foregoing considerations, I am driven to the conclusion, that in the resting nucleolus one or more threads exist, tenaciously retaining nuclear dyes and causing a skein-structure, but that the nucleolus moreover always contains a certain number of diminutive vacuoles. These vacuoles were so often observed, not only in Spirogyra but also in other plants, and the fact of their sometimes seeming to be absent is so easily explicable, that this last conclusion seems preferable to the view, that the structure of the nucleolus is not always the same and that vacuoles can sometimes be present and in other cases absent.

Further it is certain, that chromatic substance does not exist to an appreciable amount outside of the resting nucleolus and this fact is of much importance in connection with another, viz. that this same substance in the stage of the nuclear plate does exclusively appear in the twelve nuclear segments.

These facts are connected by the view, held by several authors, that by the nucleolus the chromatic substance for the segments is furnished.

The only objection against this view is, that no transitional stages are known between nucleoli and segments. But this objection is removed by my observations and from this point of view the figs.

¹⁾ l. c. p. 345.

22, 30, 31, 29, 28, 27 and 32 should be considered. I think that from these the following conclusion must be drawn. The chromatic substance, which will form the segments, at an early stage leaves the nucleolus and is transferred into the nuclear plasm. At this stage the nucleolus assumes a modified shape, getting pointed at one side and at this point the chromatic substance leaves it, appearing in the nuclear plasm as small fragments, ranged in an intermediate, achromatic thread like the beads of a neck-lace; and thus a skein, containing chromatic substance, is formed (figs. 22, 30, 31).

It seems as if the chromatic substance were squeezed from the nucleolus by an aperture ¹⁾. The thread linking the chromatic fragments together is of uncertain origin. It is possible, that it is formed from the nuclear plasm, before the chromatin leaves the nucleolus, or it could proceed from the nucleolus itself. This last supposition however is less probable, for in this case nuclei should be met with, in which only a short thread issued from the nucleolus. But though I examined a tolerable number of nuclei in this stage, some of them showing not yet much chromatic substance in the thread, this thread itself was always filling with its loops the whole nucleus as in figs. 22, 30, 31.

For this reason I think it probable, that the thread is first formed from the nuclear plasm and that afterwards the chromatin flows out into it.

Be this as it may, it has at all events been shown here, that the formation of nuclear segments from the nucleolus is accompanied by a special organisation and this seems to be no unimportant fact.

The chromatic elements in the thread now begin to fuse together at certain points and finally the thread is divided into 12 segments, each containing chromatic substance. This has been the case in figs. 27 and 28 and in the first named figure it is still apparent, that the segments possess a faintly-coloured ground-work, and that the chromatic fragments are not yet all fused together.

But in fig. 32 their fusion has become complete and 12 homo-

¹⁾ Of course it can also be supposed, that the nucleolus is emptied by suction from the nuclear plasm, but this supposition is less probable, in as far as the nucleolus does not contract or become flattened, but retains to the very last its globular shape. If this be conceded, it may be asked, what can cause the pressure within the nucleolus, by which its chromatic contents are squeezed out and I cannot refrain from mentioning the view, that such a pressure can very well be caused by an increase in size of the vacuoles, which are seen filling the nucleolus, when all chromatic substance is driven out and the segments have been formed. But though this supposition connects very well the facts recorded here, I still think, that it would be rash to discuss it more fully as a scientific hypothesis.

geneously-stained segments are to be seen. The empty nucleolus has now disappeared, but a short time before, intensely-stained specimens (fig. 27) show, that a certain number of vacuoles is present in it.

Concerning the number of segments, I already observed, that I always found, in accordance with STRASBURGER: twelve; only in one case, delineated in fig. 37: thirteen. This case is not an isolated one, as Strasburger and others mention some of these exceptions in several other plants ¹⁾.

Some words should also be said on the phenomenon of longitudinal splitting, exhibited by the nuclear segments. It is well known, that most investigators assume and do so not without cause, that the halves of segments, resulting from this splitting, separate and that, moving in different directions, each contributes towards the formation of a different daughter-nucleus, never two halves of one segment going towards the same daughter-nucleus.

This curious phenomenon has been called by FLEMMING: heteropoly. ²⁾.

Many authors are now of opinion, that in the nucleus is the seat of hereditary characters ³⁾, and the phenomena occurring in fertilisation are the chief supports of this supposition. But certainly this view can derive no inconsiderable support also from the existence of heteropoly. For if hereditary characters be concerned, it will be but natural, that the division of the nucleus into equal parts is conducted with the utmost care. And certainly heteropoly is a phenomenon, singularly adapted for obtaining such a result. From this point of view it is very important, that if heteropoly exists, it should be a generally- and well-ascertained fact. Still this is by no means the case. Most authors silently assume it, but the number of observations, on which this assumption is based, is very small. And this is not to be wondered at, for in living cells nothing of this phenomenon can be seen and the images seen in specimens, which have been treated with certain reagents and stains are mostly so very intricate, that it is impossible to draw from them more than hypothetical results.

Hence, though granting, that in a few instances heteropoly has been sufficiently proved, I still think that it is worth while to seek for more facts, proving the same and strengthening the opinion, that heteropoly universally occurs.

¹⁾ STRASBURGER. Kern- u. Zellth. p. 49.

²⁾ FLEMMING. Arch. f. mikr. Anat. Bd. 37, 1891, p. 717.

³⁾ DE VRIES. Intracellulare Pangenesis, p. 166, ss. O. HERTWIG. Die Zelle u. die Gewebe, p. 257. A. WEISMANN. Das Keimplasma. Eine Theorie der Vererbung, p. 31.

Now in *Spirogyra* the case is a very simple one, and I believe that from STRASBURGER's observations and my own it follows with certainty, that heteropoly exists here.

If, in the stage, represented in figs. 33 and 37, the segments split longitudinally and separate, without otherwise altering their relative positions, heteropoly will be the consequence. In most other plants however complex movements of the segments must take place, if heteropoly shall appear and these movements must be studied, if heteropoly shall be established.

In the case of *Spirogyra* it is therefore sufficient to prove, that no changes of place of the segments occur in the space of time between the longitudinal splitting and the separation of the halves of the segments from each other. If such changes do not take place heteropoly in *Spirogyra* is an established fact.

In this respect I think, that two figures of STRASBURGER ¹⁾ and my figures 8 and 35 are conclusive. From STRASBURGER's explanation of plate ²⁾ I infer, that both figures represent the same nucleus seen from different sides, and with my own figures this is certainly the case. From STRASBURGER's fig. 18^a and my fig. 8 it is evident, that splitting has already occurred, so that this is the moment, at which changes of place, preventing heteropoly should be seen, if they occur at all. Strasburger's fig. 18^b and my fig. 35 however show no such thing, but the very same aspect as figs. 33 and 37, where no splitting had taken place, as appears in the preliminary sketches of these nuclei, in figs. 6 and 7.

The only difference is, that the segments in fig. 35 are, in consequence of the splitting, somewhat thinner than those in figs. 33 and 37. I further direct the reader's attention to the observation of oblique sections of the nucleus, represented in fig. 9, recorded at p. 19.

My observations do not throw much additional light upon the reconstruction of the daughter-nuclei from the halves of the nuclear plate. I repeatedly saw stages, such as represented in fig. 39, the segments having fused together with their ends and forming a disc, in which apertures remained. A subsequent stage is that, delineated in fig. 16 *b* and *g* and especially in fig. 42 *a* and *b*, and this stage agrees very well with STRASBURGER's observations, cited at p. 7 and 8. But transitional stages between these two, I have as yet not been able to observe.

Lastly some remarks upon the nature and development of the

¹⁾ Kern- u. Zellth. Taf. I, figs. 18^a, 18^b.

²⁾ l. c. p. 251.

vacuoles in the nuclear spindle may be inserted. Like BEHRENS I tried to answer the question, whether, before vacuoles appear in the nuclear spindle, they be already present in the nuclear plasm and perhaps afterwards penetrate between the halves of the nuclear plate. Sometimes, drawing preliminary sketches under a low power, I saw images pointing to such an occurrence, e. g. fig. 6. But if no longitudinal sections are made, such observations procure no certainty, and in two cases, in which such sections were made, these did not show, what the preliminary sketches led to believe..

Hence I cannot say, that my observations tend towards the assumption, that the vacuoles between the daughter-nuclei are already present in the resting nucleus, but still less do they prove that a formation of new vacuoles takes place in the spindle. This question for the present remains unanswered.

For the rest I saw in nuclei, such as represented in fig. 34, that the vacuole, which first becomes visible, generally occupies a lateral position. Soon more vacuoles appear, so that in stages such as figs. 10, 11, 15 and 38 some are found, separated from each other by protoplasmic walls. But very soon one of them prevails in its dimensions (fig. 15), so that in subsequent stages (fig. 18, etc.) it seems as if only a single vacuole existed. But from figs. 15, 17 and 41 it appears, that smaller ones can still be present. These observations seem to show, that the larger vacuole pushes away the smaller ones, but at all events the possibility of a fusion of some vacuoles is by no means excluded.

The protoplasmic layer, surrounding the large vacuole, is at first very thick and remains so, even when the aequatorial dimensions of the vacuole have reached their highest point (fig. 18), but afterwards it becomes much thinner. When the vacuole becomes narrower by the protrusion of the cell-wall, its transverse section becomes nearly circular (fig. 24).

Though these observations throw no light upon the origin of the connecting vacuole, it still appears not uninteresting, that they show the presence of several vacuoles in the earlier stages.

There is still another part of our notions on this subject, which requires to be altered. As we have seen, it is believed, that through the connecting vacuole at first the spindle-fibres extend, afterwards bending outward and fusing with the surrounding protoplasm. And indeed, if no sections are made, many specimens, seen from without, are very apt to cause such an impression (figs. 10, 11, 12). But longitudinal sections show, that such is not the case and that these apparent connecting fibres are in reality the

optical sections of protoplasmic walls, separating the vacuoles. Naturally it is not impossible, that in these walls spindle-fibres occur.

Finally I will say a few words on the fine striation of the protoplasmic layer, surrounding the connecting vacuole, which is represented in fig. 42. TANGL.¹⁾ and STRASBURGER²⁾ both make mention of a similar striation and this is considered by the first author as caused, partly by the presence of spindle-fibres, partly by longitudinal folds accompanying the contraction of the vacuole.

If however fig. 42 be compared with the figures, given by these authors, it will appear, that the striation which I observed is much finer, so that here a different structure is brought to light. Moreover a comparison of the figs. 42 and 36 will show, that the spindle-fibres cannot cause this striation and the examination of figs. 42, 24 and 16 will teach, that folds of the protoplasmic layer do not exist. The nature of this striation for the present must remain unknown.

The first traces of a cell-wall I generally observed at a stage, in which a nuclear plate was visible and STRASBURGER³⁾ found the same in *Spirogyra nitida*.

The foregoing observations have brought to light some facts, especially concerning the origin of the nuclear segments, showing that the process of karyokinesis, known as very intricate, at least in *Spirogyra* is still more complicated than it was thought to be till now. It remains an open question, if in other plants, besides *Spirogyra*, similar processes occur and can be observed. For the present I will only venture to say, that I by no means share the opinion of some authors, who think that in *Spirogyra* the process of karyokinesis is essentially different from that in the higher plants.

Groningen, October 1892.

¹⁾ l. c. p. 280, 282.

²⁾ Kern- u. Zellth. p. 20.

³⁾ Zellb. u. Zellth. p. 360.

EXPLANATION OF PLATES I AND II.

Illustrating Dr. J. W. MOLL's paper "Observations on Karyokinesis in Spirogyra".

All figures are drawn from specimens of *Spirogyra crassa* Ktz., the living threads having been treated with FLEMMING's mixture of osmic, chromic and acetic acid, and the specimens stained with gentiana-violet.

PLATE I.

Fig. 1—14. Zeiss. Achr. 2 + D. Preliminary sketches (p. 11), drawn before the sections were made, from unstained threads in a medium of oil of marjoram. They correspond to the following figures, drawn at a much larger scale from the same nuclei, after the sections were made.

- | | |
|--------------------------------|----------------------------------|
| Fig. 1 = Fig. 22, cf. p. 17. | Fig. 8 = Fig. 35, cf. p. 19, 30. |
| » 2 = » 30, cf. p. 17. | » 9 cf. p. 19, 22, 30. |
| » 3 = » 31, cf. p. 18. | » 10 = Fig. 38, cf. p. 20, 31. |
| » 4 = » 27, cf. p. 18. | » 11 cf. p. 20, 31. |
| » 5 = » 32, cf. p. 18, 19. | » 12 = Fig. 39, cf. p. 21, 31. |
| » 6 = » 33, cf. p. 19, 30, 31. | » 13 = Fig. 42, cf. p. 20. |
| » 7 = » 37, cf. p. 19, 30. | » 14 = Fig. 24, cf. p. 21. |

Fig. 15. Series of consecutive, longitudinal sections, showing one large and some smaller vacuoles in the nuclear spindle. Zeiss. Achr. 2 + Apochr. 1.25. Dammar. 5 μ . Stained for 3 hours. Cf. p. 20, 21, 24, 31.

- » 16. Series of consecutive, transverse sections from two daughter-nuclei, united together by the remnant of the nuclear spindle, showing a single vacuole, which is at one side already obliterated. Between *b* and *c* a section was lost. Zeiss. Apochr. 6 + 1.25. Colophony. 5 μ . Stained for 1 hour. Cf. p. 21, 30, 32.
- » 17. Series of consecutive, longitudinal sections through a cell, showing a single large vacuole between the daughter-nuclei. In *e* and *h* the cell-walls and protoplasm are also represented. Zeiss. Achrom. 2 + Apochr. 1.25. Dammar. 5 μ . Stained for 1 hour. Cf. p. 8, 18, 15, 20, 21, 31.
- » 18. Series of consecutive, longitudinal sections through a cell, showing division in a stage between those of fig. 15 and 17. Between *a* and *b* were 2 sections, which have not been drawn, as was also the case with those subsequent to *d*. The whole number of sections from the bulged-out

C 3

spindle-figure was 10. Zeiss. Apochr. 6 + 1.25. Colophony. 5 μ . Cf. p. 20, 21, 31.

- Fig. 19. Transverse section through a whole cell. The nucleus is in the stage, represented in fig. 31, but feebly-stained. Zeiss. Apochr. 4 + 1.25. Dammar. 5 μ . Stained for 1 hour. Cf. p. 14, 15, 16, 22, 23.
- » 20. Longitudinal, median section from a thin, resting nucleus with 2 nucleoli. Zeiss. Apochr. 8 + 1.25. Dammar. 5 μ . Stained for 3 hours. Cf. p. 16, 17, 27.
 - » 21. Resting nucleus, from a transverse section. Zeiss. Apochr. 6 + 1.25. Colophony. 5 μ . Stained for 1 hour. Cf. p. 16, 17, 25, 27.
 - » 22. The largest part of a nucleus cut in two. Transverse section. The smaller part, which has not been represented here, was present in the next section and contained the portion of the nuclear membrane, missing in the figure and some parts of the thread-like structure. This nucleus is in the first stage of karyokinesis. Many thread-loops have not been delineated. Zeiss. Apochr. 4 + 1.30 (2.0). Canada-bals. 10 μ . Stained for 3 hours. Cf. fig. 1 and p. 17, 18, 21, 25, 26, 28.
 - » 23. Longitudinal, median section from a resting nucleus. Zeiss. Apochr. 8 + 1.25. Dammar. 5 μ . Stained for 3 hours. Cf. p. 16, 17.
 - » 24. Transverse section through the middle of the connecting vacuole of fig. 14. Zeiss. Apochr. 6 + 1.25. Dammar. 5 μ . Stained for one hour. Cf. fig. 14 and p. 21, 31, 32.
 - » 25. Median section through a resting nucleus, from which a series of 7 sections was got. The section next to this contained another nucleolus. Zeiss. Apochr. 8 + 1.25. Dammar. 5 μ . stained for 3 hours. Cf. p. 16, 17.
 - » 26. A detached nucleolus from a resting nucleus. Zeiss. Apochr. 6 + 1.25. Colophony. Stained for 1 hour. Cf. p. 17, 27.
 - » 27. Nucleus from a transverse section. It was divided into two parts, one of which is only represented here. The other half contained some segments and fragments of these, so that the whole number amounted to twelve, perhaps thirteen. This last number cannot be wondered at in this case, because in the same thread the nucleus, represented in fig. 37, was found. The achromatic, intermediate substance of the segments is still very apparent. Zeiss. Apochr. 4 + 1.30 (2.0). Canada-bals. 10 μ . Stained for 3 hours. Cf. fig. 4 and p. 18, 19, 25, 26, 28, 29.
 - » 28. Two consecutive sections from a nucleus nearly in the same

- stage as fig. 27, but feebly-stained. Two slices of nuclear plasm in the sections on either side of these have not been represented here. Zeiss. Apochr. 6 + 1.25. Dammar. 5 μ . Stained for 1 hour. Cf. p. 16, 18, 25, 26, 27, 28.
- Fig. 29. Nucleus from a transverse section at the same stage of karyokinesis as fig. 22, but feebly-stained. Many parts of the thread here also were left out. Zeiss. Apochr. 6 + 1.25. Colophony. 5 μ . Stained for 1 hour. Cf. p. 18, 25, 26, 27, 28.
- » 30. Nucleus from a transverse section. It is in the same stage as fig. 22 and 29, and drawn in the same manner. Zeiss. Apochr. 4 + 1.30 (2.0). Canada-bals. 10 μ . Stained for 3 hours. Cf. fig. 2 and p. 17, 18, 21, 25, 26, 28.
- » 31. Essentially the same as fig. 30. Zeiss. Apochr. 6 + 1.30 (2.0). Canada-bals. 10 μ . Stained for 3 hours. Cf. fig. 3 and p. 18, 21, 25, 26, 27, 28.

PLATE II.

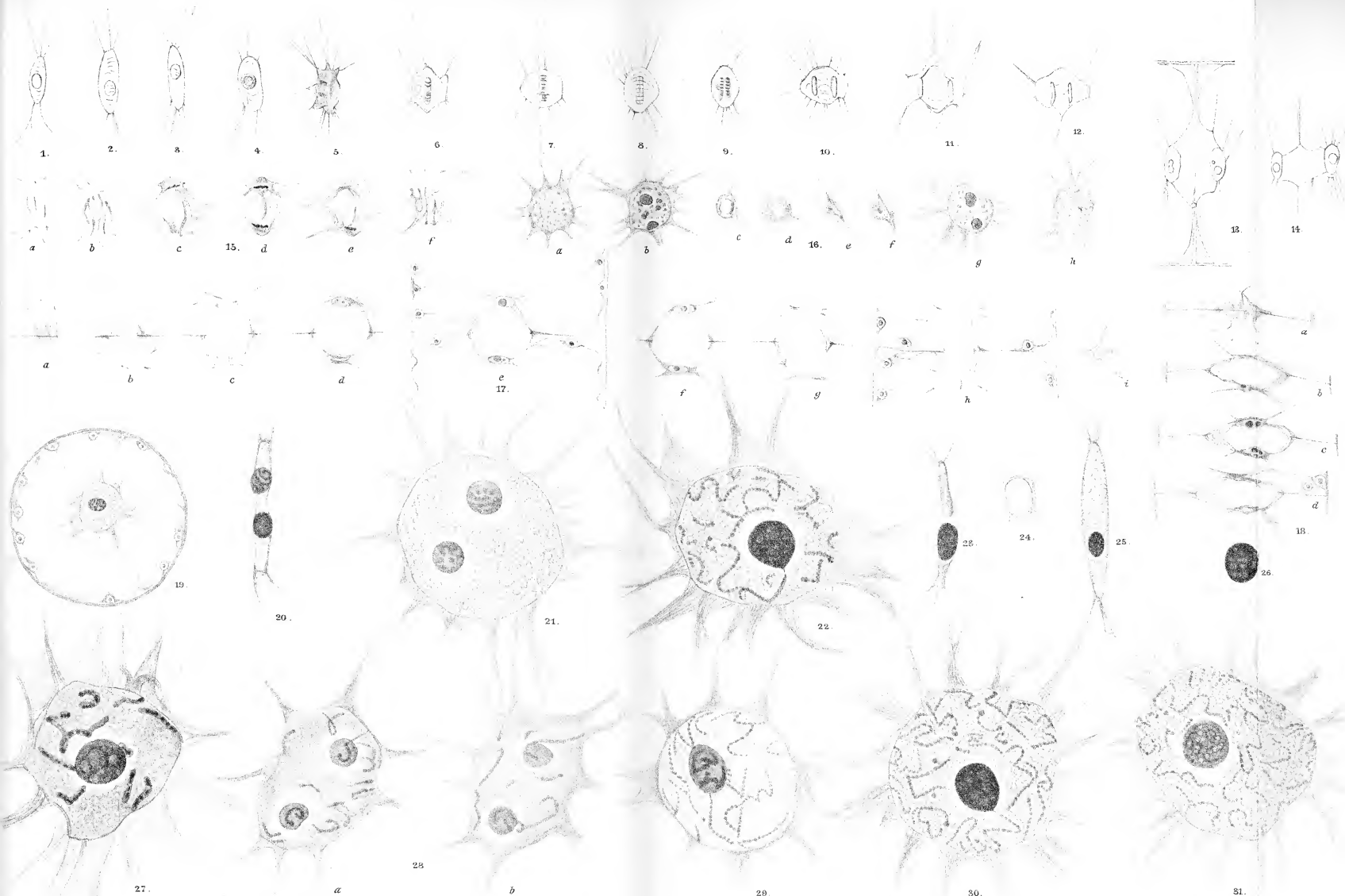
- » 32. Two consecutive, transverse sections from a nucleus at a stage more advanced than fig. 27. Twelve segments appear, one of them having been cut in two and represented by two dark points. Two slices of nuclear plasm, containing no segments and appearing in the next sections on both sides, are not reproduced here. Zeiss. Apochr. 4 + 1.30 (2.0). Canada-bals. 5 μ . Stained for 1 hour. Cf. fig. 5 and p. 18, 19, 22, 25, 28.
- » 33. Nucleus from a transverse section. Nuclear plate, before the longitudinal splitting. A slice of nuclear plasm, not represented here, was present in the next section. Zeiss. Apochr. 4 + 1.30 (2.0). Canada-bals. 10 μ . Stained for 2 or 3 hours. Cf. fig. 6 and p. 19, 22, 24, 25, 30.
- » 34. Median section through a nuclear spindle, containing 2 daughter-plates and a vacuole. Zeiss. Apochr. 6 + 1.25. Canada-bals. 5 μ . Stained for 3 hours. Cf. p. 20, 21, 31.
- » 35. Nucleus from a transverse section, both contiguous ones containing each a slice of nuclear plasm without segments. Nuclear plate after longitudinal splitting of segments. Zeiss. Apochr. 6 + 1.25. Dammar. 5 μ . Stained for 1½ hour. Cf. fig. 8 and p. 19, 22, 24, 25, 30.
- » 36. Two consecutive, longitudinal sections from a nucleus, in which the daughter-plates have separated. Zeiss. Apochr. 8 + 1.25. Dammar. 5 μ . Stained for 1 hour. Cf. p. 20, 21, 22, 32.
- » 37. Essentially the same as fig. 33, but with 13 segments. Zeiss.

Apochr. 6 + 1.30 (2.0). Canada-bals. 10 μ . Stained for 3 hours. Cf. fig. 7 and p. 19, 22, 24, 25, 29, 30.

- Fig. 38. Transverse section from the nucleus, represented in fig. 10. It was the fourth of a series of 6 sections, showing some vacuoles in the spindle. Zeiss. Apochr. 4 + 1.30 (2.0). Canada-bals. 5 μ . Stained for 2 hours. Cf. fig. 10 and p. 20, 31.
- » 39. Transverse section from the nucleus, represented in fig. 12, being the fifth of a consecutive series of six sections and containing the right-hand daughter-nucleus of fig. 12. Fusion of the segments. Zeiss. Apochr. 6 + 1.25. Colophony. 5 μ . Stained for 1 hour. Cf. fig. 12 and p. 21, 24, 30.
- » 40. Median section of a very late stage of karyokinesis. The new cell-wall has almost cut through the connecting vacuole. The next section contained the nucleolus, missing in one of the daughter-nuclei. Zeiss. Achr. 2 + Apochr. 1.25. Dammar. 5 μ . Stained for 1 hour. Cf. p. 21.
- » 41. Median section from a stage still somewhat later than fig. 40. The vacuole is cut through. Zeiss. Apochr. 8 + 1.25. Dammar. 5 μ . Stained for 3 hours. Cf. p. 21, 31.
- » 42. Four consecutive, transverse sections through the nucleus, represented in fig. 13, being the second till fifth of a series of eight sections and containing the right-hand daughter-nucleus (*a* and *b*) In figs *b*, *c*, *d* only the left part of each section has been drawn. Fine striation of the protoplasmic layer, surrounding the vacuole. Zeiss. Apochr. 4 + 1.30 (2.0). Canada-bals. 5 μ . Stained for 2 hours. Cf. fig. 13 and p. 20, 21, 24, 25, 30, 32.

The original figs. 15, 16, 17, 18, 19, 20, 23, 24, 25, 31, 36, 37 and 41 were drawn at a larger scale, so that these figures are more or less reduced; fig. 33 should have been slightly enlarged, but this was omitted.

At the proper place (p. 10) I accidentally neglected to mention, that the idea of imbedding in celloidin and paraffin is due to Kultschitzky, Zeitschr. f. wissenschaftl. Mikrosk. IV, 1887, p. 48.

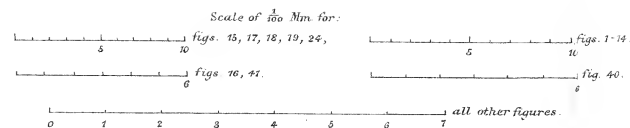
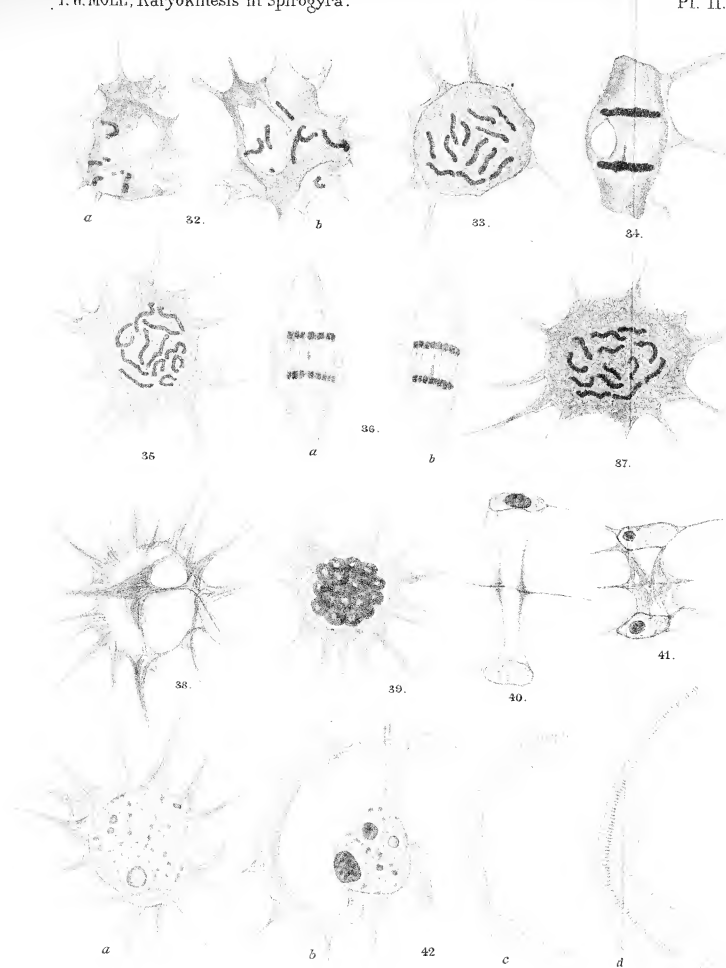


J.W. Moll del.

P.W. M. Trap impr.

Df H. W. d. G. lith.





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E.W.M. Trap impr.

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